

I Claim:

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Sub E 1. A composition of matter comprising a plurality of cells containing diverse combinations of first and second DNA sequences encoding first and second polypeptides which form heteromeric receptors, one or both of said polypeptides being expressed as fusion proteins on the surface of a cell.

2. The composition of claim 1, wherein said plurality of cells are E. coli.

MW/87 3. The composition of claim 1, wherein said heteromeric receptors selected from the group consisting of antibodies, T cell receptors, integrins, hormone receptors and transmitter receptors.

4. The composition of claim 1, wherein said first and second DNA sequences encode functional portions of heteromeric receptors.

5. The composition of claim 4, wherein said first and second DNA sequences encode functional portions of the variable heavy and variable light chains of an antibody.

6. The composition of claim 1, wherein said cell produces filamentous bacteriophage.

7. The composition of claim 6, wherein said filamentous bacteriophage are selected from the group consisting of M13, fd and f1.

Sub D 8. The composition of claim 6, wherein at least one of the encoded first or second polypeptides is expressed as a fusion protein with gene VIII.

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C 9. A kit for the preparation of vectors useful
C for the coexpression of two or more DNA sequences encoding
C polypeptides which form heteromeric receptors comprising
5 two vectors, a first vector having two pairs of restriction
sites symmetrically oriented about a cloning site which can
be combined with a second vector, having two pairs of
restriction sites symmetrically oriented about a cloning
site and in an identical orientation to that of the first
vector, wherein one or both vectors contains sequences
10 necessary for expression of polypeptides encoded by DNA
sequences inserted in said cloning sites.

10. The kit of claim 9, wherein said first and
second vectors are circular.

C 11. The kit of claim 9, wherein said ^{Coexpression} expression
C peptides is as ^a fusion proteins on the surface of a cell.

C 12. The kit of claim ¹¹ 9, wherein said cell
produces filamentous bacteriophage.

C 13. The kit of claim ¹¹ 9, wherein said filamentous
bacteriophage is selected from the group consisting of M13,
fd and f1.

14. The kit of claim 13, wherein at least one of
the DNA sequences is expressed as a fusion protein with
gene VIII.

15. The kit of claim 9, wherein said two pairs
of restriction sites are Hind III-Mlu I and Hind III-Mlu I.

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Sub E2

16. A cloning system for the coexpression of two or more DNA sequences encoding polypeptides which form a heteromeric receptor, comprising a set of first vectors having a diverse population of first DNA sequences and a 5 set of second vectors having a diverse population second DNA sequences, said first and second vectors having two pairs of restriction sites symmetrically oriented about a cloning site for containing said first and second populations of DNA sequences so as to allow only the 10 operational combination of vector sequences containing said first and second DNA sequences.

17. The cloning system of claim 16, wherein said first and second vectors are circular.

Sub F3

18. The cloning system of claim 16, wherein said heteromeric receptors selected from the group consisting of antibodies, T cell receptors, integrins, hormone receptors and transmitter receptors.

19. The cloning system of claim 16, wherein said first and second DNA sequences encode functional portions of heteromeric receptors.

20. The cloning system of claim 19, wherein said first and second DNA sequences encode functional portions of the variable heavy and variable light chains of an antibody.

21. The cloning system of claim 16, wherein said coexpression of two or more DNA sequences encoding polypeptides which form a heteromeric receptor is on the surface of cell.

Sub G4

22. The cloning system of claim 16, wherein said cell produces a filamentous bacteriophage.

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23. The cloning system of claim 22 wherein said filamentous bacteriophage selected from the group consisting of M13, fd and f1.

24. The cloning system of claim 23, wherein at least one of the DNA sequences is expressed as a fusion protein with the protein product of gene VIII.

25. The cloning system of claim 16, wherein said two pairs of restriction sites are Hind III-Mlu I and Hind III-Mlu I.

26. A plurality of expression vectors containing a plurality of possible first and second DNA sequences encoding polypeptides which form a heteromeric receptor exhibiting binding activity toward a preselected molecule, 5 said DNA sequence encoding heteromeric receptors being operatively linked to genes encoding surface proteins of a cell.

27. The expression vectors of claim 26, wherein said expression vectors are circular.

28. The expression vectors of claim 23, wherein said heteromeric receptors are selected from the group consisting of antibodies, T cell receptors, integrins, hormone receptors and transmitter receptors.

29. The expression vectors of claim 26, wherein said first and second DNA sequences encode functional portions of heteromeric receptors.

30. The expression vectors of claim 29, wherein said first and second DNA sequences encode functional portions of the variable heavy and variable light chains of an antibody.

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31. The expression vectors of claim 26, wherein
said cells produce filamentous bacteriophage.

32. The expression vectors of claim 26, wherein
said filamentous bacteriophage are selected from the group
consisting of M13, fd and f1.

33. The expression vectors of claim 32, wherein
at least one of the encoded ~~first~~ or second polypeptides is
expressed as a fusion protein with gene VIII.

34. A method of constructing a diverse
population of vectors capable of expressing a diverse
population of heteromeric receptors, comprising:

5 (a) operationally linking to a first vector
a first population of diverse DNA
sequences encoding a diverse population
of first polypeptides, said first
vector having two pairs of restriction
sites symmetrically oriented about a
cloning site;

10 (b) operationally linking to a second
vector a second population of diverse
DNA sequences encoding a diverse
population of second polypeptides, said
second vector having two pairs of
restriction sites symmetrically
oriented about a cloning site in an
identical orientation to that of the
first vector; and

15 (c) combining the vector products of step
20 (a) and (b) under conditions which
allow only the operational combination
of vector sequences containing, said
first and second DNA sequences.

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35. The method of claim 34, wherein said first and second vectors are circular.

36. The method of claim 34, wherein said heteromeric receptors are selected from the group consisting of antibodies, T cell receptors, integrins, hormone receptors and transmitter receptors.

37. The method of claim 34, wherein said first and second DNA sequences encode functional portions of the variable heavy and variable light chains of an antibody.

38. The method of claim 34, wherein said expression of a diverse population of heteromeric receptors is on the surface of a cell.

39. The method of claim 37, wherein said cell produces a bacteriophage.

40. The method of claim 39, wherein said filamentous bacteriophage is selected from the group consisting of M13, fd and f1.

41. The method of claim 34, wherein at least one of said first or second DNA sequences is expressed as a gene VIII fusion protein.

42. The method of claim 34, wherein said two pairs of restriction sites are Hind III-Mlu I and Hind III-Mlu I.

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43. The method of claim 34, wherein said combining step further comprises:

- 5
- (C1) restricting said first vector with a restriction enzyme recognizing one of the restriction sites encoded in said two pairs of restriction sites;
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- (C2) restricting said second vector with a different restriction enzyme recognizing the second restriction site encoded in said two pairs of restriction sites;
- (C3) digesting the 3' ends of said restricted first and second vectors with an exonuclease; and
- 15
- (C4) annealing said first and second vectors.

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44. A method for selecting a heteromeric receptor exhibiting binding activity toward a preselected molecule from a population of diverse heteromeric receptors, comprising:

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(a) operationally linking to a first vector a first population of diverse DNA sequences encoding a diverse population of first polypeptides, said first vector having two pairs of restriction sites symmetrically oriented about a cloning site;

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(b) operationally linking to a second vector a second population of diverse DNA sequences encoding a diverse population of second polypeptides, said second vector having two pairs of restriction sites symmetrically oriented about a cloning site in an identical orientation to that of the first vector;

(c) combining the vector products of step (a) and (b) under conditions which allow only the operational combination of vector sequences containing said first and second DNA sequences.

(d) introducing said population of combined vectors into a compatible host under conditions sufficient for expressing said population of first and second DNA sequences; and

(e) determining the heteromeric receptors which bind to said preselected molecule.

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45. The method of claim 44, wherein said first and second vectors are circular.

46. The method of claim 44, wherein said heteromeric receptors are selected from the group consisting of antibodies, T cell receptors, integrins, hormone receptors and transmitter receptors.

47. The method of claim 44, wherein said first and second DNA sequences encode functional portions of heteromeric receptors.

48. The method of claim 47, wherein said first and second DNA sequences encode functional portions of the variable heavy and variable light chains of an antibody.

49. The method of claim 44, wherein said expression of a diverse population of heteromeric receptors is on the surface of a cell.

50. The method of claim 49, wherein said cell produces a filamentous bacteriophage.

51. The method of claim 50, wherein said filamentous bacteriophage is selected from the group consisting of M13, fd and fl.

52. The method of claim 51, wherein at least one of said first or second DNA sequences is expressed as a gene VIII fusion protein.

53. The method of claim 44, wherein said two pairs of restriction sites are Hind III-Mlu I and Hind III-Mlu I.

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54. The method of claim 44, wherein said combining step further comprises:

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(C1) restricting said first vector with a restriction enzyme recognizing one of the restriction sites encoded in said two pairs of restriction sites;

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(C2) restricting said second vector with a different restriction enzyme recognizing the second restriction site encoded in said two pairs of restriction sites;

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(C3) digesting the 3' ends of said restricted first and second vectors with an exonuclease; and

(C4) annealing said first and second vectors.

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- 55. A method for determining the nucleic acid sequences encoding a heteromeric receptor exhibiting binding activity toward a preselected molecule from a diverse population of heteromeric receptors, comprising:

- 5 (a) operationally linking to a first vector
10 a first population of diverse DNA
15 sequences encoding a diverse population
20 of first polypeptides, said first
25 vector having two pairs of restriction
30 sites symmetrically oriented about a
cloning site;

(b) operationally linking to a second vector a second population of diverse DNA sequences encoding a diverse population of second polypeptides, said second vector having two pairs of restriction sites symmetrically oriented about a cloning site in an identical orientation to that of the first vector;

(c) combining the vector products of step (a) and (b) under conditions which allow only the operational combination of vector sequences containing said first and second DNA sequences.

(d) introducing said population of combined vectors into a compatible host under conditions sufficient for expressing said population of first and second DNA sequences;

- 5 () determining the heteromeric receptors which bind to said preselected molecule;
- (f) isolating the nucleic acid sequences encoding said first and second polypeptides; and
- (g) sequencing said nucleic acid sequences.

56. The method of claim 55, wherein said first and second vectors are circular.

57. The method of claim 55, wherein said first heteromeric receptors selected from the group consisting of antibodies, T cell receptors, integrins, hormone receptors and transmitter receptors.

58. The method of claim 55, wherein said first and second DNA sequences encode functional portions of heteromeric receptors.

59. The method of claim 58, wherein said first and second DNA sequences encode functional portions of the variable heavy and variable light chains of an antibody.

60. The method of claim 55, wherein said expression of a diverse population of heteromeric receptors is on the surface of a cell filamentous bacteriophage selected from the group consisting of M13, fd and f1 and at least one of said first or second DNA sequences is expressed as a gene VIII fusion protein.

61. The method of claim 55, wherein said cell produces filamentous bacteriophage.

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62. The method of claim 61, wherein said filamentous bacteriophage is selected from the group consisting of M13, fd and f1.

63. The method of claim 62, wherein at least one of said first or second DNA sequences is expressed as a gene VIII fusion protein.

64. The method of claim 50, wherein said two pairs of restriction sites are Hind III-Mlu I and Hind III-Mlu I.

65. The method of claim 50, wherein said combining step further comprises:

5 (C1) restricting said first vector with a restriction enzyme recognizing one of the restriction sites encoded in said two pairs of restriction sites;

10 (C2) restricting said second vector with a different restriction enzyme recognizing the second restriction site encoded in said two pairs of restriction sites;

(C3) digesting the 3' ends of said restricted first and second vectors with an exonuclease; and

15 (C4) annealing said first and second vectors.

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DW/H

66. A vector comprising two copies of a gene encoding a filamentous bacteriophage coat protein, one copy of said gene capable of being operationally linked to a DNA sequence encoding a polypeptide of a heteromeric receptor wherein said DNA sequence can be expressed as a fusion protein on the surface of said filamentous bacteriophage or as a soluble polypeptide.

67. The vector of claim 66, wherein said two copies of said gene encode substantially the same amino acid sequence but have different nucleotide sequences.

68. The vector of claim 66, wherein said one copy of said gene is expressed on the surface of said filamentous bacteriophage.

69. The vector of claim 66, wherein said bacteriophage coat protein is M13 gene VIII.

70. The vector of claim 66, wherein said vector has substantially the same sequence as that shown in Figure 2 (SEQ ID NO: 1).

DW/H

71. A vector comprising sequences necessary for the coexpression of two or more inserted DNA sequences encoding polypeptides which form heteromeric receptors and two copies of a gene encoding a filamentous bacteriophage coat protein, one copy of said gene capable of being operationally linked to one of said two or more inserted DNA sequences wherein said DNA sequence can be expressed as a fusion protein on the surface of said filamentous bacteriophage or as a soluble polypeptide.

72. The vector of claim 71, wherein said two copies of said gene encode substantially the same amino acid sequence but have different nucleotide sequences.

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73. The vector of claim 71, wherein said one copy of said gene is expressed on the surface of said filamentous bacteriophage.

74. The vector of claim 71, wherein said bacteriophage coat protein is M13 gene VIII.

75. The vector of claim 71, wherein said vector has substantially the same sequence as that shown in Figure 6 (SEQ ID NO: 5).

add
B3

add
C4